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Comparative Gametophyte Morphology of Selected Species of the Family Polypodiaceae

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ABSTRACT.—Gametophyte morphology and growth habit of seven epiphytic species of six genera in the Polypodiaceae are described. In addition to the *Drynaria*-type of early development, depicted previously in most of this family and expressed in all species observed, the *Ceratopteris*-type, *Adiantum*-type, and *Aspidium*-type also occurred in some of the species observed. All species observed were long-lived, clone-forming, and without gemmae. The long-lived, clone-forming habit increases the space and time occupied by gametophytes, and thus enhances the probability of interaction with other gametophytes and the probability of intergametophytic mating in epiphytic habitats.

Gametophyte morphologies vary greatly beyond the standard heart shapes usually depicted in textbooks (Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Atkinson, 1973). These variations include the ability to grow indeterminately and to branch, so that perennial gametophyte clones of considerable size may be produced. In some families, gametophytes also have the ability to reproduce themselves vegetatively by dispersable gemmae (Farrar, 1993a, b; Raine, 1994; Dassler, 1995). Gemma production and/or clonal growth have been observed primarily in epiphytic species.

Although most of the variation in gametophyte morphology is among epiphytic species, most of the information known on reproductive biology is from research on terrestrial ferns. Epiphytic ferns exist in an environment very different from that of terrestrial species. The gametophytes of tropical epiphytic species are often found within dense bryophyte mats (Dassler, 1995). In such habitats, interaction between gametophytes via chemicals (antheridiogen) and sperm transfer may be significantly hindered relative to gametophytes of terrestrial ferns on smooth surfaces (Dassler, 1995). Gametophyte persistence and expansion by clone formation may help to overcome these difficulties and promote reproduction in the aerial habitat. In order to assess the significance of morphological diversity to fern reproduction in different habitats, further knowledge of the morphology of gametophytes in epiphytic species is needed.

Most species of Polypodiaceae are epiphytic (Lellinger, 1985). We observed the morphology of the gametophytes of seven epiphytic species from this family. Results are interpreted with respect to modes and mechanisms of the species' reproductive biology.

MATERIALS AND METHODS

The species observed are listed in Table 1. Spores obtained from fertile fronds (Chiou and Farrar, 1994) were stored in a refrigerator at about 5°C.

TABLE 1. Polypodiaceae species studied, their distribution, and collection source. All vouchers are at ISC.

Species	Distribution	Collection
Campyloneurum angustifolium (Sw.) Fée	Florida, Mexico, Central and South America	ISU Greenhouse, source unknown
Campyloneurum phyllitidis (L.) C. Presl	Florida, Mexico, West Indies, Central and South America	ISU Greenhouse, source unknown
Lepisorus thunbergianus (Kaulf.) Ching	China, Taiwan, Japan, Philippines, Hawaii	Hawaii, *Farrar 92-8-16-4*
Microgramma heterophylla (L.) Wherry	Florida, West Indies	ISU Greenhouse, source unknown
Phlebodium aureum (L.) J. Sm.	Florida, Mexico, West Indies, Central and South America	Florida, *Chiou 14342*
Phymatosorus scolopendria (Burm.) Pic. Serm.	Ryukyus, Taiwan, Hainan, Malaya, Hawaii	Hawaii, *Farrar 92-8-20-1*
Polypodium pellucidum Kaulf.	Hawaii (endemic)	Hawaii, *Farrar 92-8-19-3*

Voucher specimens were deposited in the Ada Hayden Herbarium (ISC) of the Department of Botany at Iowa State University.

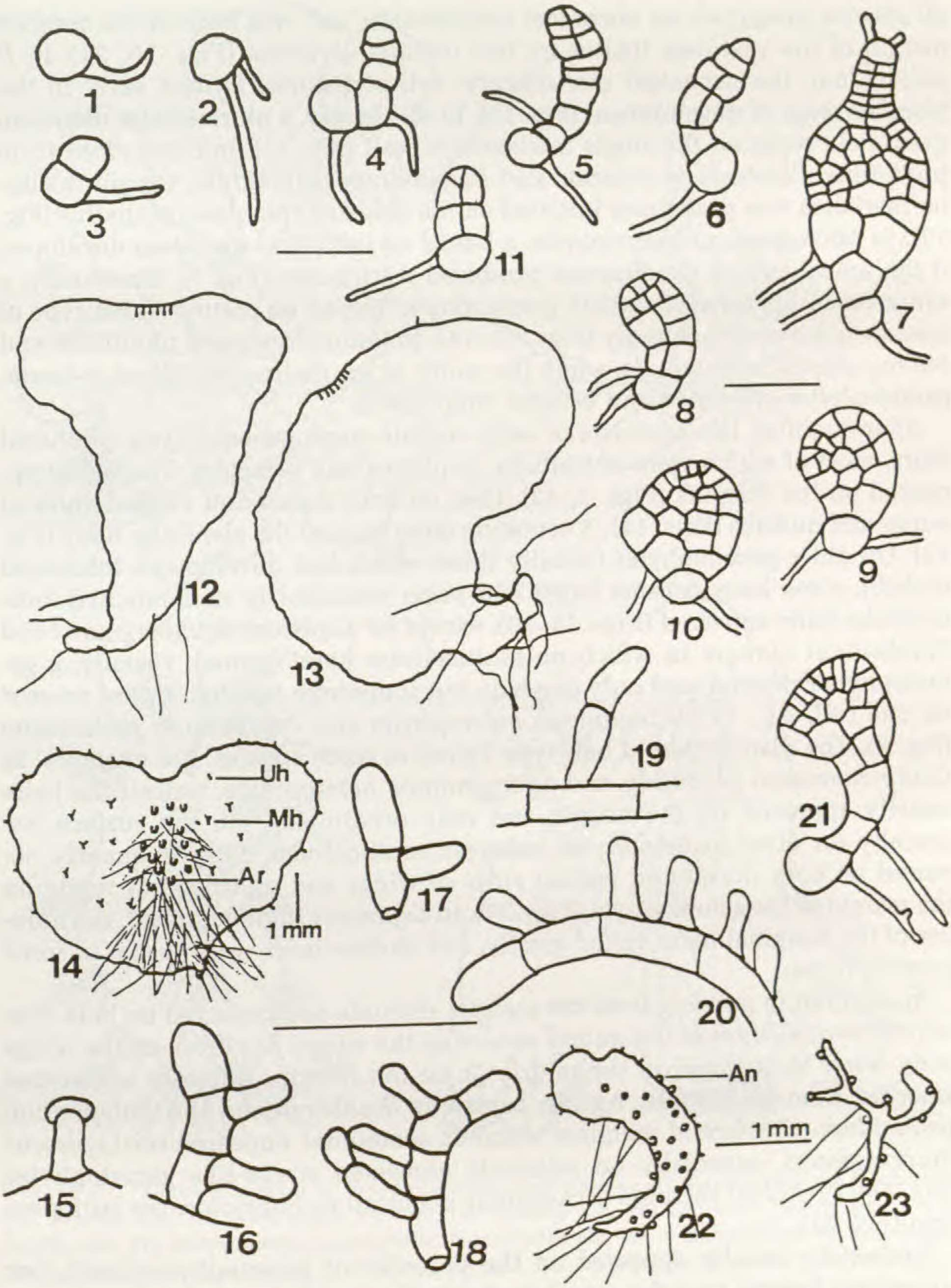
Spores were sown on 1% agar-solidified media that contained Bold's macronutrients (Bold, 1957), Nitsch's micronutrients (Nitsch, 1951), and a trace of ferric chloride (Farrar, 1974). Cultures were maintained under continuous, white fluorescent illumination of 2000–3000 lux. Temperature was maintained at 20–24°C.

For microscopic observation, gametophytes were removed from culture and mounted in water. Drawings were made with the aid of a drawing tube. Photographs were taken through compound and dissecting microscopes or a 55 mm macrolens.

RESULTS

In multispore cultures, spores divided first into a basal cell that remained inside the spore wall and a rhizoidal cell that elongated to form a slender, nonseptate rhizoid (Fig. 1). The basal cell underwent a second division (Fig. 3), resulting in a protonemal cell that subsequently produced a filament by serial transverse divisions (Fig. 4). Occasionally, a second rhizoid appeared before the protonemal cell in *Campyloneurum phyllitidis* and *Polypodium pellucidum* (Fig. 2). The lengths of filaments varied from two to eight cells. Additional rhizoids also emerged from filament cells below the apical cell of the protonema. Filament growth was terminated by formation of a division parallel to the axis of the filament (longitudinal division), usually first in the apical cell, but occasionally beginning in other cells of the filament (Figs. 5, 6). Sometimes all the filament cells except the basal cell eventually divided longitudinally (Fig. 8). Subsequent divisions and expansion of filament cells formed a broad spathulate prothallus (Fig. 9).

The development of the meristem varied among species. In most plants of



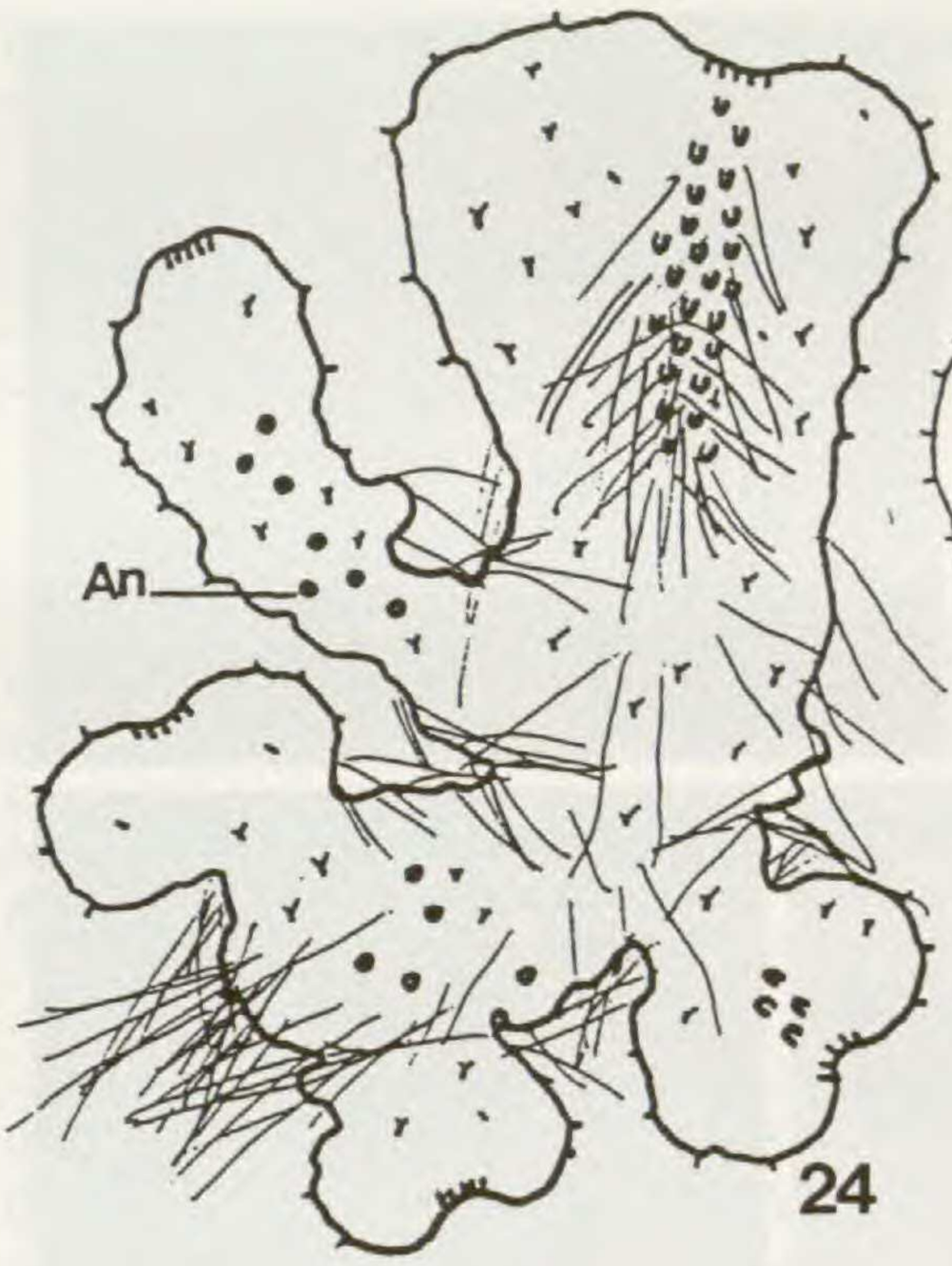
FIGS. 1–23. Development and morphology of Polypodiaceae gametophytes. 1–4) *Campyloneurum phyllitidis*. 5–10) *Microgramma heterophylla*. 11) *Polypodium pellucidum*. 12–18) *Campyloneurum phyllitidis*. 19–21) *Phymatosorus scolopendria*. 22–23) *Polypodium pellucidum*. An = antheridium. Ar = archegonium. Mh =multicellular hair. Uh = unicellular hair. Scale bar = 0.1 mm unless otherwise indicated.

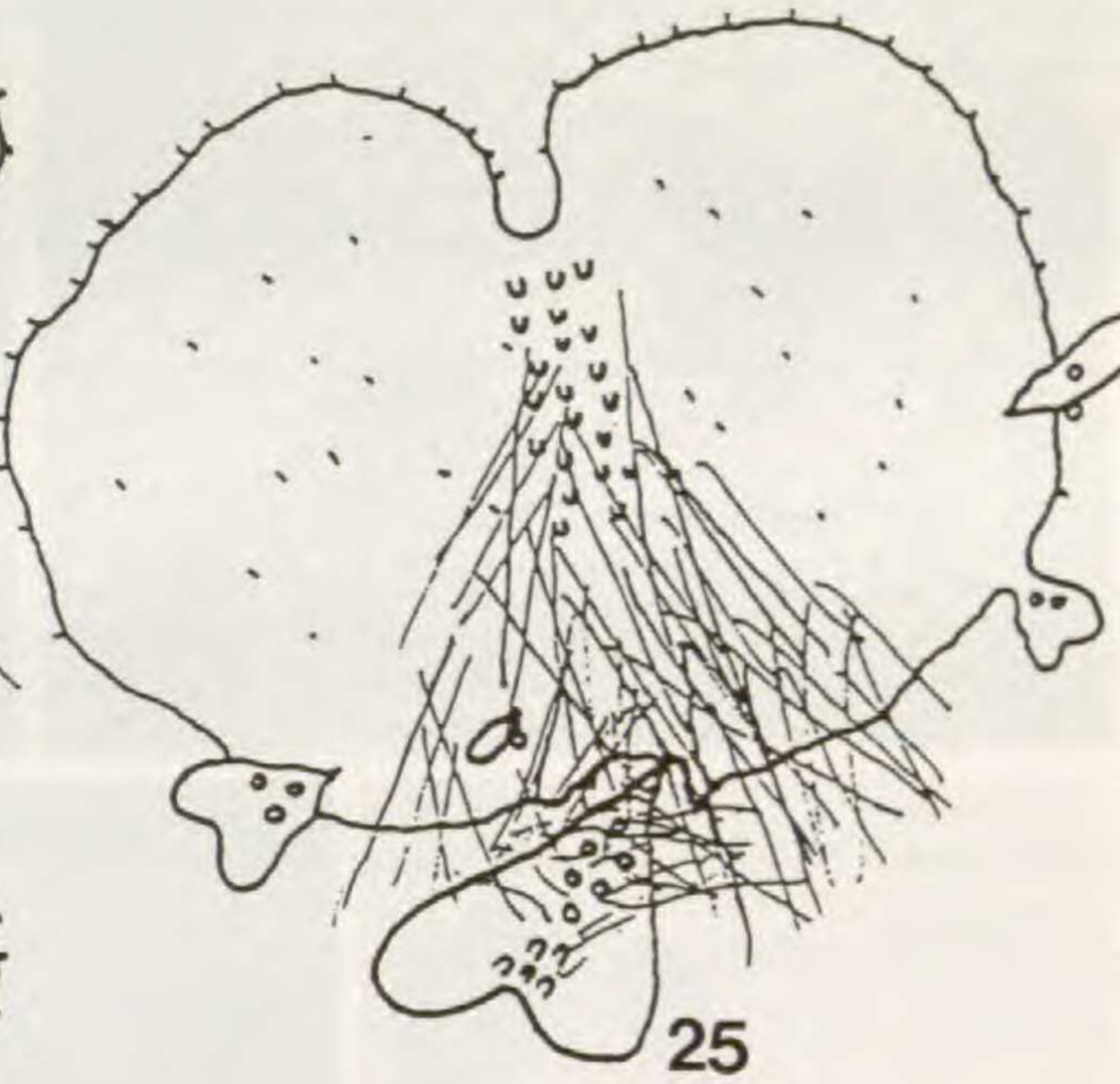
all species examined, an obconical meristematic cell was built at the anterior margin of the spatulate thallus by two oblique divisions (Figs. 10, 21). In *P. pellucidum,* the obconical meristematic cell sometimes formed early in the biseriate stage of development (Fig. 11). In all species, a pluricellular meristem eventually replaced the single meristematic cell (Fig. 12). In *Campyloneurum phyllitidis, Phlebodium aureum,* and *Polypodium pellucidum,* the pluricellular meristem was sometimes initiated on the side of a spatulate prothallus (Fig. 13). In *Microgramma heterophylla,* a lateral or sublateral meristem developed if the apical cell of the filament produced a trichome (Fig. 7). Eventually, a symmetrical (or nearly) cordate gametophyte formed no matter which type of meristem developed initially (Figs. 12, 14). In *Campyloneurum phyllitidis* and *Microgramma heterophylla,* when the width of the thallus was about 3–4 mm, gametophytes elongated and became strap-like.

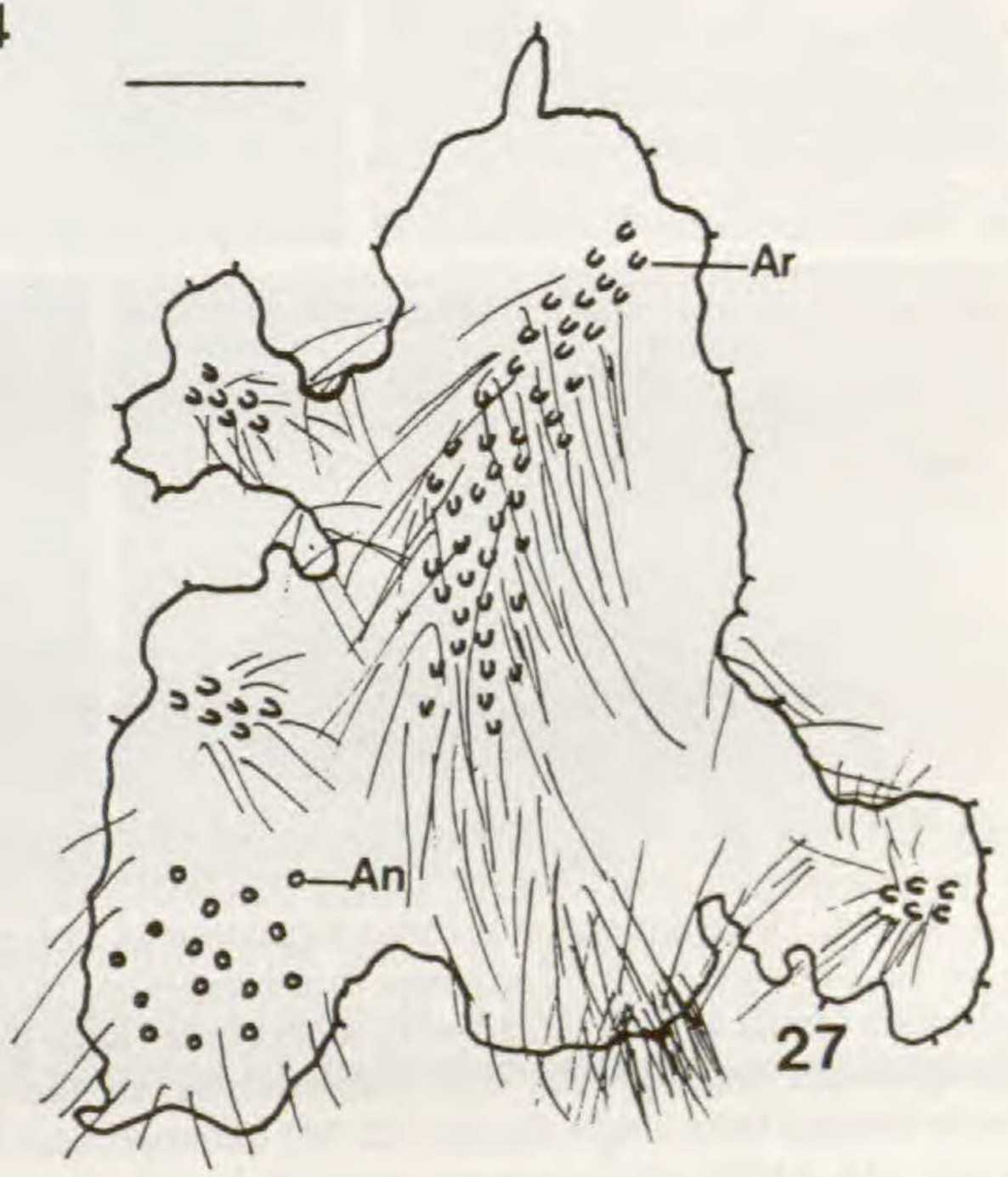
After attaining late spatulate or early cordate stage, gametophytes produced hairs, most of which were unicellular, papillate, and secretory. These first appeared on the margins (Figs. 7, 13), then on both dorsal and ventral sides of wings and midribs (Figs. 14). A secretion often capped the glandular hairs (Fig. 15). On older gametophytes (usually those which had developed a thickened midrib), some hairs became larger and some branched or nonbranched multicellular hairs appeared (Figs. 15–20), except for *Lepisorus thunbergianus* and *Phlebodium aureum* in which no multicellular hairs formed. Usually, a gametophyte cell produced only one hair, but sometimes two hairs were present on one cell, e.g., in *Microgramma heterophylla* and *Polypodium pellucidum* (Fig. 7). The distribution of hair type varied in some species. For example, in *Campyloneurum phyllitidis* and *Microgramma heterophylla,* unicellular hairs usually appeared on the margin and only occasionally on the surface, especially on older gametophytes, whereas multicellular hairs frequently occurred on both dorsal and ventral sides of wings and midribs, but rarely on the margin of the gametophyte (Figs. 14). In *Lepisorus thunbergianus,* the number of the marginal hairs varied greatly, and surface hairs were absent in some gametophytes.

In addition to growing from the margin, rhizoids also occurred on both dorsal and ventral sides of the midrib as well as the wings, but those on the wings were fewer than those on the midrib or on the margin. Rhizoids sometimes emerged from archegonia, e.g., in *Lepisorus thunbergianus* and *Polypodium pellucidum.* Clusters of marginal rhizoids sometimes appeared on *Lepisorus thunbergianus,* especially on relatively elongated strape-like gametophytes (Fig. 26). Branched rhizoids occasionally occurred in *Phymatosorus scolopendria* (Fig. 21).

Antheridia usually appeared on the posterior of gametophyte plants, but sometimes formed near the apical meristem if a midrib had not formed, or on the margin, especially on small irregular-shaped gametophytes (Figs. 22, 23) that usually occurred later in the culture period or in areas of high gametophyte density. Archegonia always were distributed along the midrib behind the meristem (Fig. 14, 24–27). In *Lepisorus thunbergianus,* archegonia were sometimes located along a discontinuous midrib (Fig. 28). In *Lepisorus thun-*



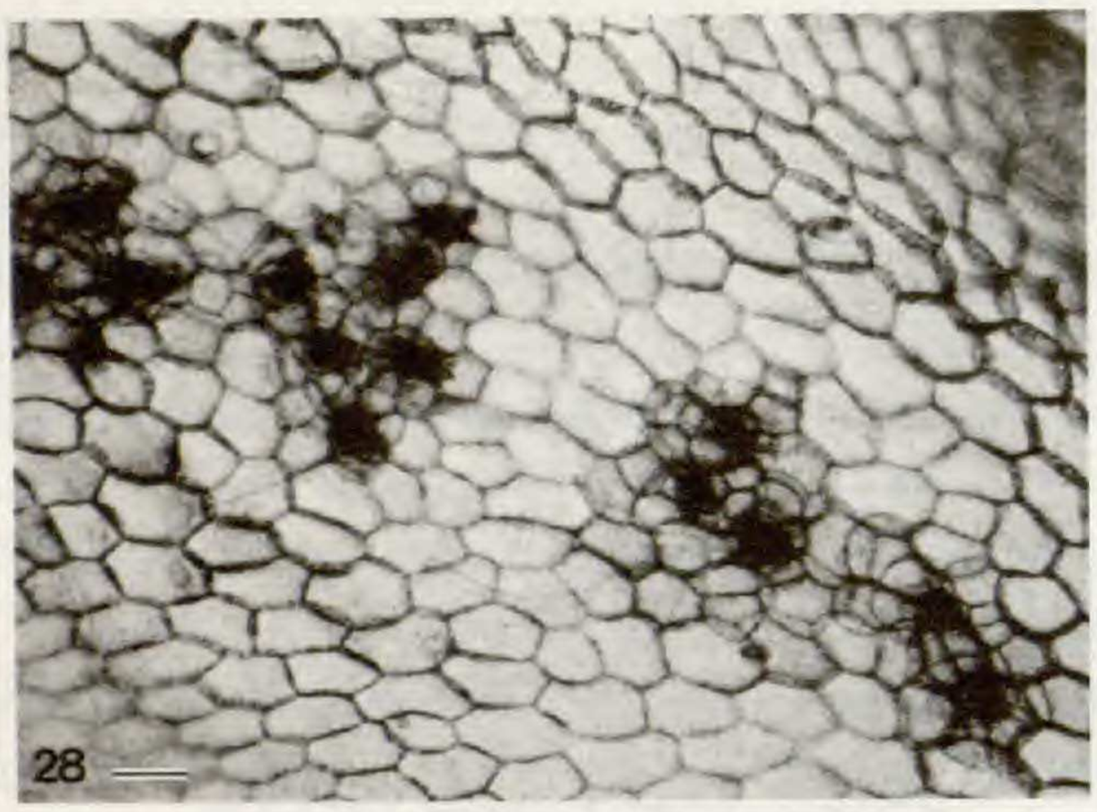


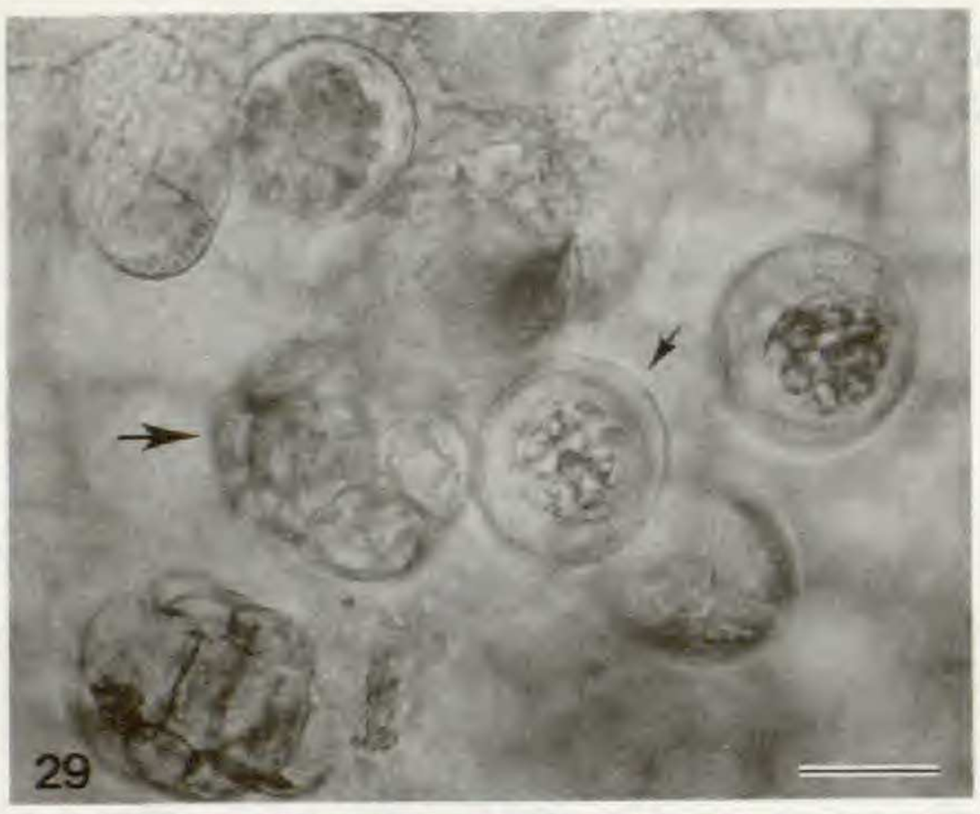


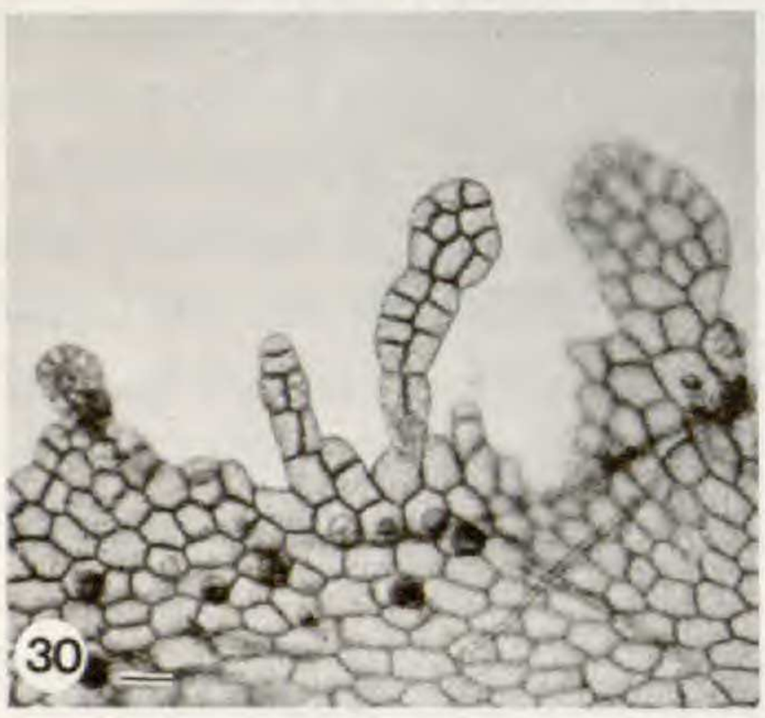
FIGS. 24–27. Clone formation by Polypodiaceae gametophytes. 24) *Campyloneurum phyllitidis,* four months old. 25) *Phlebodium aureum,* two months old. 26) *Lepisorus thunbergianus,* six months old. 27) *Microgramma heterophylla,* four months old. An = antheridium. Ar = archegonium. Scale bar = 1 mm.

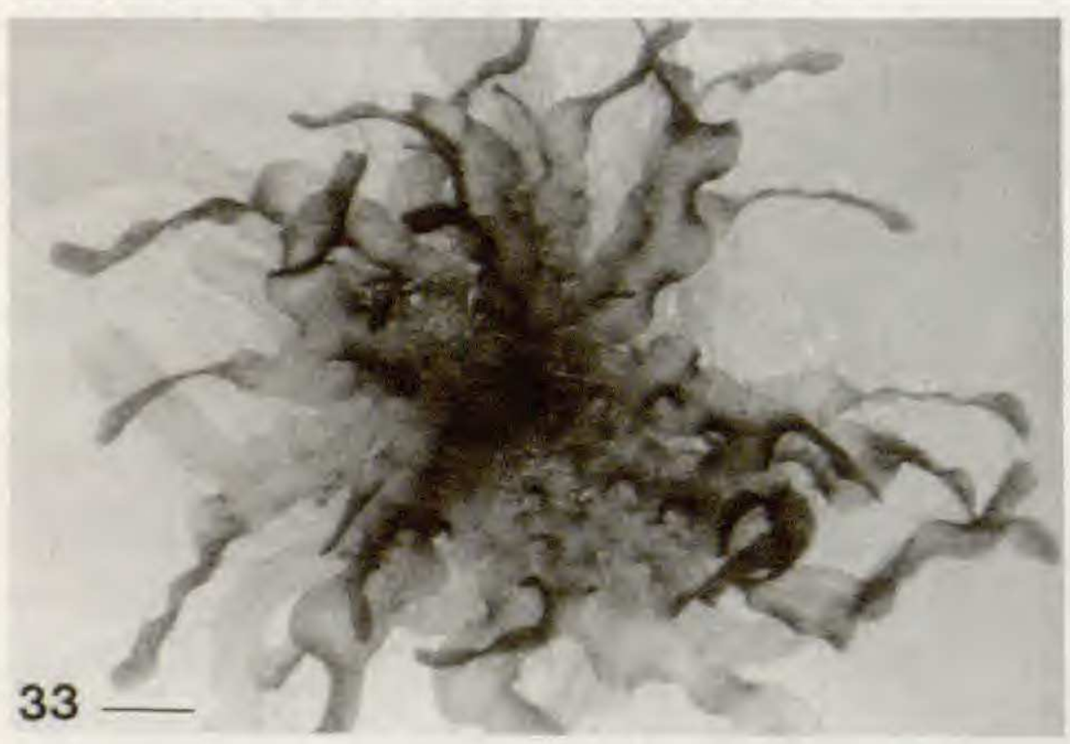
bergianus and *Phlebodium aureum,* antheridia became mixed with archegonia on the older gametophytes (Fig. 29).

When gametophytes reached their maximum width or nearly so, branching occurred. Two kinds of branching were observed: 1) a broad segment of mar-









FIGS. 28–34. Morphology and clone formation in Polypodiaceae gametophytes. 28–31) Gametophyte morphology. 28, 29) *Lepisorus thunbergianus;* 28) archegonia on discontinuous midribs; 29) antheridia (small arrow) mixed with archegonia (large arrow); 30) proliferations on the margin of *Microgramma heterophylla*; 31) proliferations on the surface of *Phlebodium aureum*. 32–34) Clones formed from single spores. 32, 33) *Microgramma heterophylla*; 32) two years old; 33) eight months old. 34) *Campyloneurum angustifolium*, six month old. Scale bar = 0.1 mm for Figs. 28–31; 1 cm for Fig. 32, 1 mm for Figs. 33, 34.

ginal cells became a pluricellular meristem that produced a branch (Figs. 24, 27); 2) one or a few cells produced a proliferation similar to a prothallus that elongated and expanded to some degree, then developed a pluricellular meristem (Fig. 25). The second type was common to gametophytes of all species studied, whereas the first type was seen only in *Campyloneurum angustifolium, Campyloneurum phyllitidis, Lepisorus thunbergianus,* and *Microgramma heterophylla* (Table 2). In *Lepisorus thunbergianus,* the principal meristem also

TABLE 2. Summary of gametophyte habitat, morphology, and growth habits in seven species of Polypodiaceae. Abbreviations—Habitat: E = epiphytic, H = hemiepiphytic, R = rupestral, T = terrestrial. Development type: Ad = *Adiantum*-type, As = *Aspidium*-type, C = *Ceratopteris*-type, D = *Drynaria*-type (Nayar and Kaur, 1971). Mature form: C = cordate-thalloid, S = strape-like (Nayar and Kaur, 1971). CL = clone-formation. Branch type: 1 = from a broad segment of marginal cells, 2 = from one or a few cells of the margin or surface.

Species	Habitat	Development type	Mature form	Branch type	Longevity
Campyloneurum angustifolium	E, R	D	C, CL	1, 2	Long-lived
Campyloneurum phyllitidis	E, R	C, D	C, S, CL	1, 2	Long-lived
Lepisorus thunbergianus	E, R	D	C, S, CL	1, 2	Long-lived
Microgramma heterophylla	E, R	As, D	C, S, CL	1, 2	Long-lived
Phlebodium aureum	E, R, T	C, D	C, CL	2	Long-lived
Phymatosorus scolopendria	E, H, R, T	D	C, CL	2	Long-lived
Polypodium pellucidum	E, R, T	Ad, C, D	C, CL	2	Long-lived

divided to form dichotomous branches (Fig. 26). Branches of the second type developed either from the margin (Fig. 30) or from the surface (Fig. 31) of gametophytes. Multiple branching from a single original gametophyte eventually yielded a mat of of overlapping thalli (Figs. 32–34). New proliferations subsequently produced gametangia. Gametophyte clones continued growing and after two years had produced multiple sporophytes from different thalli of the clone. Clonal expansion continued after sporophyte initiation through growth and branching of non-sporophyte-producing apices (Fig. 35, 36).

DISCUSSION

The early gametophyte development of Polypodiaceae has been classified previously as primarily *Drynaria* type, rarely *Kaulinia*-type or *Aspidium*-type (Nayar and Kaur, 1971). In this study, the *Drynaria*-type appeared in every species. An additional type (*Ceratopteris*-type) occasionally occurred in *Campyloneurum phyllitidis, Phlebodium aureum,* and *Polypodium pellucidum*. In *Polypodium pellucidum,* the *Adiantum*-type and in *Microgramma heterophylla,* the *Aspidium*-type also appeared (Table 2). Thus, early development seems to be somewhat plastic and perhaps of limited usefulness as a character for systematic purposes.

Gametophytes of most, perhaps all, fern species are capable of regenerating new prothalli from older ones (Atkinson and Stokey, 1964; Nayar and Kaur, 1971). Likewise, all gametophytes have potential for continued growth of the original meristem in the absence of sporophyte production. However, a significant quantitative difference exists in species' expression of these capabilities. Gametophytes of terrestrial species, when grown in sufficient light and space, generally remain as single cordate individuals over usual periods of laboratory culture (2–6 months). This is also the case for gametophytes of terrestrial species we have observed in the wild for up to one year (Peck et al., 1990). In contrast, gametophytes of epiphytic Vittariaceae and Hymenophyl-

FIGS. 35 and 36. Several sporophytes (arrows) derived from gametophyte clones produced from single spores. 35) *Campyloneurum phyllitidis,* 2.5 year old. 36) *Phymatosorus scolopendria,* 1.5 year old. Scale bar = 0.5 mm for Fig. 35; 1 cm. for Fig. 36.

laceae branch at an early age in culture (<1 month) as part of normal maturation, and wild gametophytes of these families and of Grammitidaceae are nearly always branched (Farrar, 1993a, b; Dassler, 1995). This seemingly programmed development of branching also characterizes all the Polypodiaceae species in this study.

Gametophytes of many epiphytic species (especially Vittariaceae and Hymenophyllaceae) also differ from those of terrestrial species in that cells derived from the apical meristem expand primarily along their longitudinal axis, creating an elongated strap- or ribbon-like thallus, whereas gametophytes of most terrestrial species expand primarily in width, creating the familiar butterfly- or heart-shaped thallus. Both types of expansion are displayed among the species of Polypodiaceae. Mature gametophytes of *Christiopteris, Colysis, Dendroglossa, Kaulinia, Leptochilus,* and *Paraleptochilus* have been described as ribbon-like with a broad, rounded meristematic apex, those of *Selligua* and some species of *Lepisorus* as elongated and strap-like with an apical notch meristem, and most Polypodiaceae species as cordate-thalloid with an apical notch meristem (Nayar and Kaur, 1971). In our study, gametophytes of *Campyloneurum phyllitidis, Lepisorus thunbergianus* and *Microgramma heterophylla* exhibited strap-like growth as mature plants, whereas individual thalli of *Campyloneurum angustifolium, Phlebodium aureum, Phymatosorus scolopendria,* and *Polypodium pellucidum* remained cordate, except when they grew in very high density or under very low light intensity where they became slender and strap-like. Both types of growth permitted multiple and continuous production of sporophytes from different thalli of clones derived from a single spore. Thus, although morphology of the mature thallus varies within the Polypodiaceae, persistent growth and clone formation may be characteristic of the family.

Starch-gel enzyme electrophoresis studies by many researchers indicate that most terrestrial sporophytes of homosporous ferns are produced through outcrossing (e.g., Soltis and Soltis, 1992). Assuming increased difficulty of sperm

transfer in the bryophyte dominated reproductive habitats of epiphytic ferns, one might expect a high degree of selfing in epiphytic species. To the contrary, enzyme electrophoresis studies have indicated that epiphytic ferns also tend to be outcrossing (Masuyama et al., 1987; Ranker, 1992; Haufler, 1995). Could the gametophyte morphology of epiphytic ferns be an adaptation promoting outcrossing? Long life and clone formation have been hypothesized to be of adaptive value both in competing with bryophytes in the epiphytic habitat and in increasing the opportunity for gametophyte interaction (Dassler, 1995). Dassler stated that this growth habit ". . . allows a fern gametophyte to physically migrate to a microsite favorable for growth and reproduction, as well as to survive until favorable microsite conditions occur, and to persist until the arrival of a second migrant", thus facilitating outcrossing. The perennial clone-forming habit also assures continued presence of gametes due to production of gametangia on new proliferations. If we consider antheridiogen function, the promotion of gametophyte interaction by clone-formation and long-life is even more significant, and includes the potential for interaction with previously buried spores (Haufler and Welling, 1994; Chiou, 1996).

Numerous descriptions of gametophyte morphology by A. G. Stokey, L. R. Atkinson, B. K. Nayar and many others (Atkinson and Stokey, 1964; Nayar and Kaur, 1971; Atkinson, 1973) have characterized and differentiated fern taxa according to spore germination, early development type, mature form, trichome type, gametangium type and position, etc., and combinations of these characters. Although these studies have established the potential contribution of gametophyte characters to fern systematics, little attention has heretofore been given to the functional basis of gametophyte form. The association of certain gametophyte characteristics, i.e. those contributing to the formation of long-lived clones, with certain habitats, i.e. tropical epiphytic, calls for careful examination of gametophyte characters with consideration of possible parallel evolution of morphological characters among taxa.

Acknowledgments

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